

RESEARCH ARTICLE

Calendula Oil Associated Dexibuprofen Nanoformulation Transdermal Patches: Formulation and Characterization

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ABSTRACT

Objectives: To prepare and characterize the nanoparticle-based topical formulations of dexibuprofen in combination with calendula oil to enhance the topical anti-inflammatory effect.

Methods: Chitosan and tri poly phosphate (TPP) were used to prepare nanoparticles developed utilizing the ionotropic gelation technique and analyzed by scanning electron microscope (SEM) and the ZS. Solvent casting was used to prepare the transdermal medication delivery system for the chitosan nanoparticle formulation.

Results: Results revealed that nanoparticles were between the sizes of 267.3 nm. This particle size may enhance the drug's solubility and penetration through the skin. These patches containing chitosan nanoparticles were produced utilizing polymer combinations - HPMC, chitosan, and PEG400 with tween80 as plasticizers. Accompanying the rise in hydrophilic polymer content came an increase in the rate of medication release via the patch.

Conclusion: Transdermal patch - F3 released the drug reasonably well.

Keywords: Nanoparticles, Transdermal patches, Dexibuprofen, Calendula oil.

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INTRODUCTION

According to World Health Organization (WHO) statistics, the primary class – non-steroidal anti-inflammatory drugs (NSAIDs) medicines, the second most often used drug in the world. For two centuries, they have been utilizing them to reduce inflammation and pain.¹ Ibuprofen (IBU), a derivative of propionic acid, was the most preferred NSAID for therapy out of all of them.² However, one of the commonly recommended substitutes for ibuprofen lately is dexibuprofen (DIBU), the S-isomer of the therapeutically effective racemic ibuprofen. This compound is claimed to have superior safety and be more pharmacologically active and tolerated than ibuprofen due to the greater concentration of active S enantiomer.³ Despite being thought to be safer than ibuprofen, it nevertheless

has a few significant adverse effects, such as sour stomach, light-headedness, liver, and central nervous system (CNS) effects when taken orally. Here, the medicine has been first transformed into nanoparticles and then packaged into patches for convenience of application to improve topical penetration and increase its safety profile.⁴ With this method of drug delivery, the medication can relieve symptoms by penetrating the local subcutaneous tissue while avoiding potential negative effects on the rest of the body.⁵

Due to its many topical uses, calendula oil is one of the essential oils that has been used traditionally. Since its primary purpose is to reduce inflammation, a novel combination of dexibuprofen and calendula oil has been proposed to improve the topical action of dexibuprofen. Therefore, the goal of

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this study is to create topical patches based on nanoparticles that contain dexibuprofen in combination with calendula oil and then assess them for investigations involving *in-vitro* characterization.^{6,7}

MATERIALS AND METHODS

Materials

Dexibuprofen (SOLARA Active Pharma Sciences, Chennai), calendula oil (Vedha oil), glacial acetic acid 2% (Merck specialist), tripolyphosphate ((SOLARA Active Pharma Sciences, Chennai), HPMC (Himedia, Bombay), chloroform (Sisco research), PEG400 (Nice chemicals), methanol (Himedia, Bombay), Tween 80 (Merck specialist), chitosan (Thermo Fischer), potassium dihydrogen orthophosphate (Fischer inorganics), sodium hydroxide pellets (Thermo Fischer Scientific). These are the various ingredients used for the manufacture of nanoparticle-based transdermal patches.

Methods

Pre-formulation studies of dexibuprofen and calendula oil nanoparticle

The ultimate aim of this study is to optimize dosage from manufacturing conditions and characterize each component. To enable wise excipient selection, the study often examines the interactions between medicinal components and excipients.

Differential scanning calorimetry studies

Differential scanning calorimetry (DSC) study was conducted on dexibuprofen, calendula oil, placebo, and medication plus placebo. The temperature ranged from 0 to 600°C, and the heating rate was 10°C/min with a 10 mL/min inert nitrogen flow rate. Calendula oil, dexibuprofen, placebo, and medication with placebo samples were weighed and administered at 10 mg, 10 mL, 6, and 7.3 mg, respectively. Every sample had a unique code, such as D for the dexibuprofen sample, CO for the calendula oil sample, DP2 for the medication plus placebo, and P2 for the placebo. The aluminum pans were a reference, and the sample was loaded into standard platinum pans. The aluminum pans had corresponding weights of 10 mg and 10 mL. The degree of melting of each component in the sample was ascertained.⁸ The melting point of each component in the sample was identified, interpreted, and analyzed and the values were recorded.

Fourier-transform infrared spectral studies

By directing the beam towards the test sample, fourier-transform infrared (FTIR) calculates the number of spectra and wavelengths whereby infrared (IR) radiation gets absorbed by the specimen. To allow infrared light to flow through, just a small portion of the material must be removed. Certain specimens must be treated with reflectance techniques without inflicting damage to them. Spectroscopy analyses were performed for dexibuprofen, calendula oil, placebo, and medicine coupled with placebo using a Bruker, Inc. instrument. Spectroscopy studies were analyzed using standard wave numbers and interpreted.⁹⁻¹¹

Formulation of dexibuprofen and calendula oil nanoparticles

About 2 g of glacial acetic acid were blended with a 100 mL of purified water to create a 2% acetic acid solution. After adding 0.02 g of chitosan to the mixture. The mixture was violently agitated until it became clear and turbidity-free. The pH of 4 to 5 of the solution is also found. Before proceeding, add methanol as q.s. and 0.1 g of dexibuprofen to a second beaker. Next, pour the solution into the beaker with the glacial acetic acid and chitosan at a concentration of 2%. Stir until a clear solution is achieved. After agitating the solution slowly for an hour and keeping the beaker containing it in the ice bath, gradually add one cc of calendula oil to the mixture. In a different beaker, make a tripolyphosphate (TPP) solution (0.2 g of TPP in 1-mL of deionized water). Pour the TPP solution into the beaker with the medicine. After adding two drops of tween 80 to the mixture, stir it for 30 minutes using a magnetic stirrer. Spinning the drug-containing tube at 1500 rpm for 30 minutes is necessary after adding the solution to the centrifugation tube. Following centrifugation, the clear solution tube was divided, and the nanoparticles that had accumulated at the tube's bottom were taken out for the experiment that followed.¹²

Evaluation prepared nanoparticles

The characterization of nanoparticles was done using Zeta sizer Nano ZS, zeta potential Nano ZP (Malvern instruments), and scanning electron microscopy (SEM).

- *Determination of particle size*

Using DLS, the Malvern particle size and zeta potential Analyzer measures particle and molecule sizes from less than a nanometer to several microns, and electrophoretic light scattering measures zeta potential. The investigation of nanoparticle dispersed particle size using this device has been published.¹³

- *Determination of zeta potential*

At the point of the slipping plane, electrical potential is known as the zeta potential, which serves as the barrier that divides liquid and flows easily away from objects that are stationary on the surface. The technical term for the electrokinetic potential in colloidal dispersions is zeta potential. Zeta potential is a prominent and readily quantifiable indicator of colloidal dispersions' stability. The magnitude of the zeta potential indicates the intensity of electrostatic repulsion among neighboring, similarly charged particles in a dispersion. This equipment has been used to study the zeta potential of nanoparticles, and the findings have been published.

- *Scanning electron microscope studies of nanoparticles*

SEMs are a type of electron microscope in which pictures of the sample are created by scanning a surface with a focused electron beam. The interaction between the atoms and electrons in the sample results in a range of signals that provide information on the surface topography and composition of the sample. When the beam of electrons scans in a grid-like pattern, its position and the intensity of the received signal are combined to generate a picture. By capturing SEM pictures,

the size and morphological properties of the nanoparticles were assessed.¹⁴

Formulation of nanoparticles into transdermal patches

Following the formulation of the nanoparticles, 5 g of HPMC was combined with PEG400 (2.5 mL) and enough chloroform to create a smooth paste consistency (Table 1). The combination was then sonified in a sonicator for 30 minutes. Following sonification, take a petri plate, lubricate it with glycerol, and pour the sonicated solution into the dish. Finally, cover the petri dish with an inverted funnel and let it dry for a full day.¹⁵

Evaluation Studies for Transdermal Patches

Thickness test for the patches

Vernier caliper scale was utilized at three separate locations to determine the patch’s thickness and it should be uniform at each site. The thickness of the patch was calculated.

Folding endurance test

This study is used to determine the flexibility of the patch. The patch was folded at the specific area 300 times continuously. This shows the folding endurance and the results were recorded.^{16,17}

Determination of percentage moisture content in patches

The patches were individually weighed and kept in the dehumidifier for about 24 hours with fused calcium chloride. After this procedure, it should be reweighed.

$$\text{Percentage moisture content} = \frac{(\text{initial weight} - \text{final weight})}{\text{final weight}} \times 100$$

Determination of percentage moisture uptake

These patches were stored at the dehumidifier for about 24 hours with potassium chloride at 84% relative humidity. After this procedure, it should be reweighed.

$$\text{Percentage Moisture uptake} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$$

Drug content of patches

The 2 × 2 area film was weighed, dissolved in an appropriate solvent (methyl alcohol), diluted with phosphate buffer saline (pH 7.4), and percolated. After performing various serial dilutions, it was found by the UV method by measuring the absorbance at 222 nm using the standard curve.

Flatness test of the patches

Three strips of the patches were cut at all the sides. By calculating the %constrictions, length variation is quantified. Total flatness is equal to 0% restriction.

Disintegration test of the patches

The disintegration test of the patches was done by cutting the patches into a 2x2 area and then soaked in the deionized water continuously by placing the patch in a wire gauze at the center. The time taken to disintegrate completely in water and the readings were recorded.

In-vitro diffusion study

The cellophane membrane was previously soaked for 6 to 7 hours in deionized water using in diffusion study. The patch was cut into a 2 × 2 cm² area.¹⁸ Then, the patch was placed at the center of the membrane, tightly tied with the thread and freely hung into the beaker containing buffer using a burette stand. At the bottom of the stand, the beaker of 250 mL was placed with 100 mL of buffer. Below the beaker, the magnetic stirrer was placed and stirred with the temperature control of 37°C to resemble body temperature. The time was recorded and the samples were taken at 10, 20, 30, 40, 50, 60 minutes intervals. About 5 mL of the sample was taken in the beaker and 5 mL of the buffer was replaced with the syringe. Then, the drug diffusion study was analyzed using a UV-visible spectrophotometer, and the intensity of absorption at 222 nm was measured.

RESULTS AND DISCUSSION

Differential Scanning Calorimetry

The drug’s physical alterations and compatibility with its excipients were examined using the DSC analysis.

Figure 1 shows an endothermic peak at 105°C, which corresponds to the melting point of the drug dexibuprofen.¹⁹ Hence, through Figure 1 it was confirmed that the taken sample

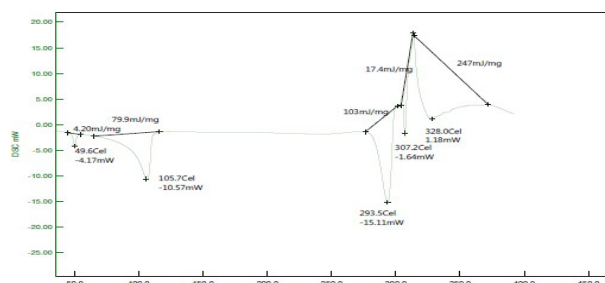


Figure 1: Pure dexibuprofen’s DSC thermogram

Table 1: Formulation of transdermal patches

Ingredients	Quantities for surface area (54 cm ²)		
	Batch 1	Batch 2	Batch 3
Nanoparticle formulation	equals 100 mg of dexibuprofen and 1-mL of calendula oil	equals 100 mg of dexibuprofen and 1-mL of calendula oil	equals 100 mg of dexibuprofen and 1-mL of calendula oil
HPMC (gm)	1.25	2.5	5.0
PEG400 (mL)	2.5	2.5	2.5

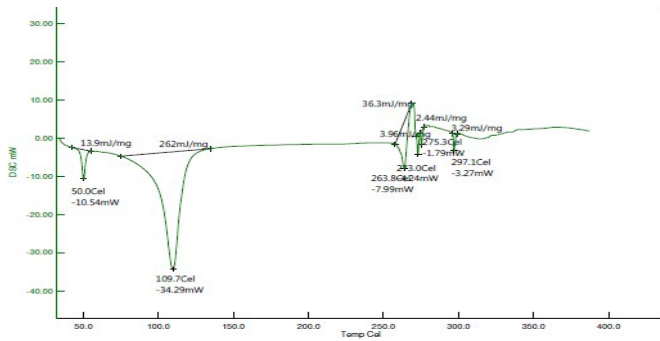


Figure 2: Calendula oil's DSC thermogram

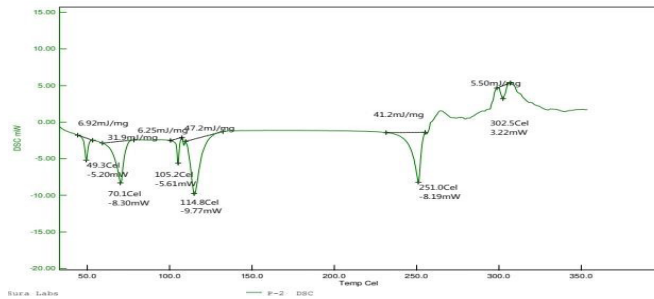


Figure 3: The placebo combination of nanoparticles' DSC thermogram

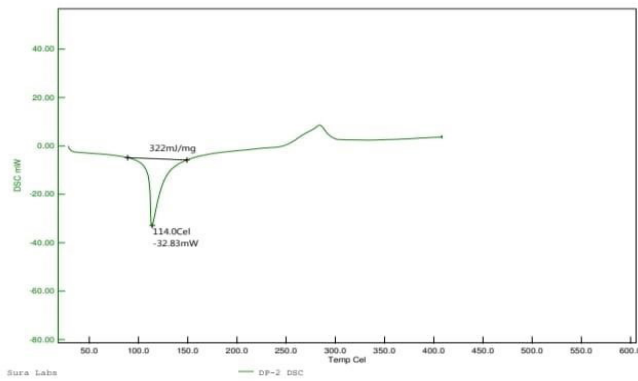


Figure 4: The DSC thermogram of the nanoparticle mixture of drug and placebo nanoparticle

is dexibuprofen. Figure 2 shows the DSC thermogram of calendula oil. The literature review says that the chosen oil was calendula essential oil, as it possesses an endothermic peak at 49.6°. Figure 3 showed the placebo mixture of the formulation and showed endothermic peak at 251 (HPMC), and 70.1°C (Chitosan). Figure 4 shows the comprehensive DSC containing APIs and a placebo of nanoparticles. This thermogram reveals a slight shift of the endothermic peak towards the right to the higher temperature (114°C). This shift may be due to the physical interaction between the API and the placebo during the processes of nanonization.

FTIR Spectral Studies

In Figure 5, the FTIR spectrum of dexibuprofen exhibits distinct features such as the aromatic CH₃ stretch at 744 cm⁻¹,

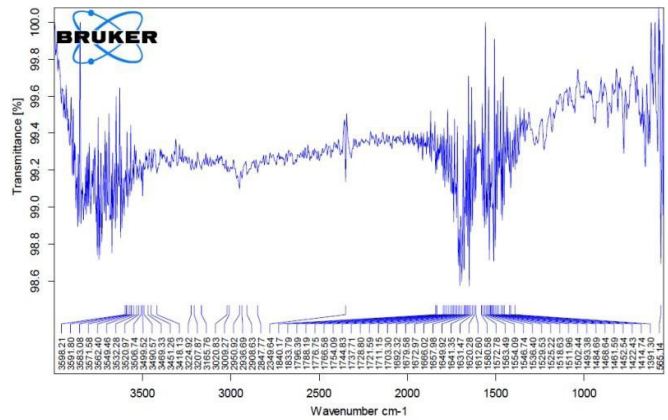


Figure 5: Dexibuprofen's FTIR spectrum

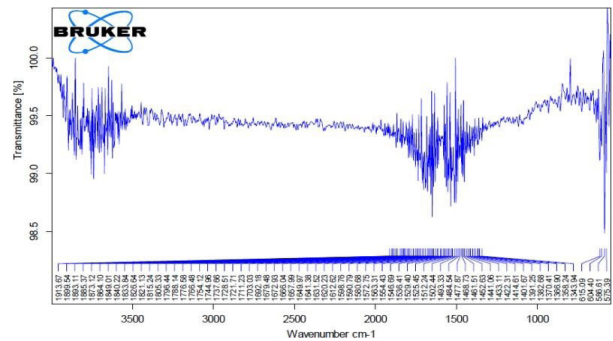


Figure 6: Calendula oil's FTIR spectrum

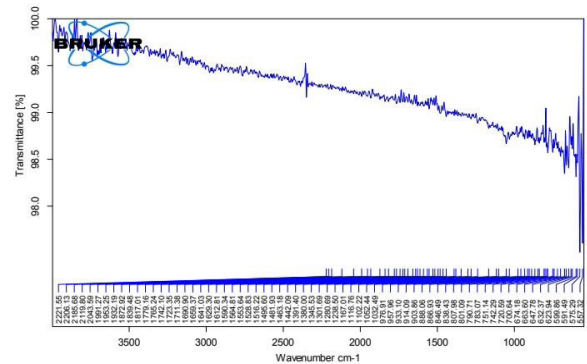


Figure 7: A placebo combination of nanoparticles' FTIR spectra

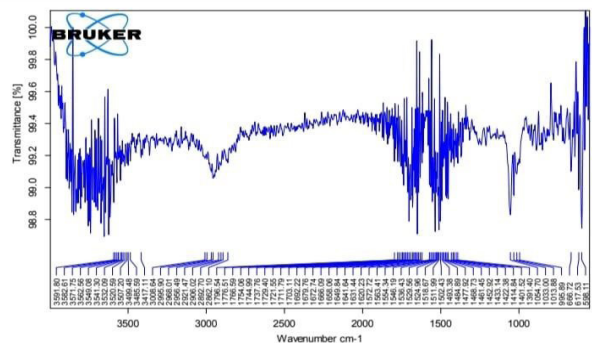


Figure 8: FTIR spectra of drugs with excipients of nanoparticles

the OH stretch at 1068 cm^{-1} , the C=C benzene stretch at 1465 cm^{-1} , and the distinctive C=O stretch at 1705 cm^{-1} . FTIR spectra of calendula oil is shown in Figure 6. In the drug placebo (nanoparticle) combination spectra (Figures 7 and 8), all of these groups were located at 1707, 742, 1068, and 1462 cm^{-1} .²⁰ These spectra indicate drug has no interaction with oil as well as with other excipients in the formulations. The FTIR study was done for compatibility study with dexibuprofen. FTIR spectra of the drug dexibuprofen showed the characteristic absorbance for C=O (COOH) peak at around 1744 approx. A characteristic of dexibuprofen was its absorbance for the OH group peak, which was around 3224. The approximate absorbance of the C--O(S) group is 1391. Further, demonstrating that the medication does not interact with either the oil or the excipients is the C=O peak, which is visible in the combination at a range of 1724.

The developed analytical method is simple and accurate for the estimation of dexibuprofen. Using phosphate buffer saline with a pH of 6.8 as a blank, the drug samples were examined using UV spectroscopy at absorption maxima of 222 nm. Drug complies with Beer's law limit between 10 and 80. Furthermore, this approach is easy to use and affordable, making it suitable for regular quality monitoring of dexibuprofen in pharmaceutical formulations.

Preparation and Evaluation of Calendula Oil With Dexibuprofen Nanoparticles

Particle size analysis studies of nanoparticles

A nanoparticle's average size is 267.3 nm. This dimension may improve a drug's solubility and skin penetration (Figure 9).²¹

Zeta potential analysis studies of nanoparticles

The zeta potential for the prepared nanoparticle formulation is found to be 69.4 mV. Hence, the prepared formulation (Figure 10) is macroscopically stable.²²

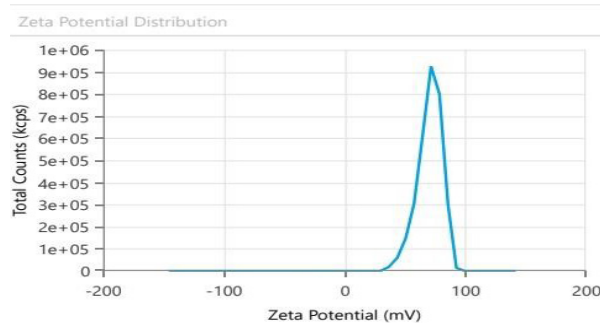


Figure 10: Zeta potential curve of nanoparticles

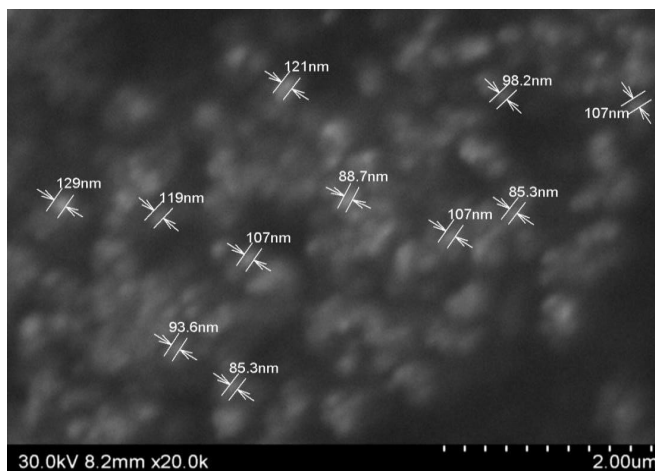


Figure 11: SEM photograph of nanoparticles

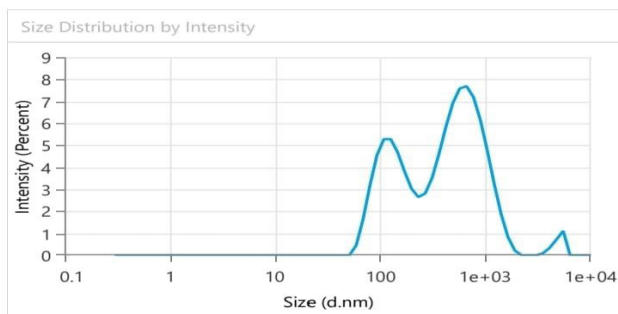


Figure 9: Particle size distribution of nanoparticles



Figure 12: Morphology of calendula reinforced dexibuprofen transdermal patch

Table 2: Evaluation of transdermal patches

Batch No.	Thickness (mm)	Folding endurance (times)	Moisture content (%)	Moisture uptake (%)	Drug content (%)	Flatness (%)	Disintegration test (min)
1.	0.6 ± 0.2	52 ± 10	4.27 ± 1.5	28.94 ± 5	75.723 ± 6	85.72 ± 3.2	3.2 ± 0.3
2.	0.5 ± 0.2	55 ± 7	6.07 ± 2.0	32.02 ± 4	88.1341 ± 7	89.12 ± 3.0	2.5 ± 0.7
3.	0.5 ± 0.2	50 ± 9	3.56 ± 0.5	24.89 ± 10	89.8641 ± 5	91.86 ± 4	3.6 ± 0.5

*n = 3

Scanning electron microscopic studies of nanoparticles

SEM studies revealed the shape of the nanoparticles is nearly spherical (Figure 11) and these results further confirmed the nano size of the formulation.²³

Evaluation of prepared nanoparticle-based transdermal patches

The developed nanoparticle-based DIBU patches (Figure 12) are soft and transparent to white in color with smooth texture.

Evaluation reports of nanoparticle-based transdermal patches showed satisfactory values (Table 2) and hence the methodology used to prepare patches is reliable and can be routinely used.

CONCLUSION

For use in *in-vitro* assessment investigations, three batches of nanoparticle-associated patches have been effectively synthesized and characterized. The produced nanoparticle-based patches demonstrated repeatable quality attributes, according to the study’s findings. In summary, the produced formulations based on calendula nanoparticles and dexibuprofen show promise as dosage forms with good medicinal qualities. Nonetheless, it is necessary to carry out both *in-vivo* and *ex-vivo* experiments to verify their therapeutic effectiveness. For the treatment of acute pain and localized inflammation, these formulations could be investigated in the future as an oral administration replacement.

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